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ARTÍCULO ORIGINAL

Feeding success of *Lutzomyia evansi* (Diptera: Psychodidae) experimentally exposed to small mammal hosts in an endemic focus of *Leishmania chagasi* in northern Colombia

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Lutzomyia evansi is the vector of *Leishmania chagasi* in northern Colombia. Differences in feeding success were revealed, when this phlebotomine sand fly was fed on five species of small mammal hosts from an endemic focus of visceral leishmaniasis. In each trial, 50 female sand flies were provided access to similar-sized depilated areas of the hind foot of each of 44 individual mammals and allowed to feed for 30 minutes. The number of engorged sand flies was counted at the end of each trial and compared among host species by analysis of variance and Tukey's multiple comparisons test. Sand flies fed least successfully on *Sciurus granatensis*, a common squirrel in the endemic area. It has not been found infected with *L. chagasi*. Intermediate numbers of sand flies engorged on *Heteromys anomalus* and *Zygodontomys brevicauda*, but these two mammals have not been found infected with *L. chagasi* and are not expected to be important in transmission. Sand flies fed most successfully on *Didelphis marsupialis* and *Proechimys canicollis*. These are the two most abundant mammals in the endemic area and frequently are infected. Results provided further evidence that these two species are the wild mammals with the greatest impact on transmission of *L. chagasi* in northern Colombia.

Key words: Phlebotomines, *Leishmania*, reservoirs, Colombia, attraction.

Éxito de la alimentación de *Lutzomyia evansi* (Diptera: Psychodidae) expuestos experimentalmente a reservorios mamíferos pequeños en un foco endémico de *Leishmania chagasi* en el norte de Colombia

Un método sencillo de laboratorio reveló diferencias en el éxito de alimentación de *Lutzomyia evansi*, el vector de *Leishmania chagasi* en el norte de Colombia, cuando se alimentó sobre cinco especies de pequeños mamíferos de un foco endémico de leishmaniasis visceral, en los que éstos podrían actuar como reservorios. En cada ensayo, a 50 flebótomos hembra se les permitió alimentarse durante 30 minutos sobre un área similar de piel depilada de la pata posterior en 44 mamíferos. El número de flebótomos alimentados se comparó entre especies a través de un análisis de varianza y de la prueba de Tukey de comparaciones múltiples. Los flebótomos escasamente se alimentaron sobre *Sciurus granatensis*, una ardilla común en el área endémica que no se ha encontrado infectada con *Leishmania chagasi*. En otros dos mamíferos que hasta el presente han sido negativos para *L. chagasi* (*Heteromys anomalus* y *Zygodontomys brevicauda*), los flebótomos se alimentaron en bajos números. En cambio, los flebótomos se alimentaron en altas proporciones sobre *Didelphis marsupialis* y *Proechimys canicollis*, los dos mamíferos más abundantes en el área endémica y que se hallan infectados con *L. chagasi*. Los resultados aportaron evidencia adicional que estas dos especies de mamíferos silvestres serían una fuente de sangre común para los flebótomos y que, por lo tanto, pueden tener gran impacto sobre la transmisión de *L. chagasi* en el norte de Colombia.

Key words: flebótomos, *Leishmania*, reservorios, Colombia, atracción.

Transmission of vector-borne disease agents involves many factors, of which host-vector interactions are of capital importance. Studies on such interactions should consider not only the abundance of hosts and their attractiveness to vectors but also the subsequent feeding success of vectors.

In the New World, field studies on the biting behavior of sand flies (*Lutzomyia* spp.), vectors of *Leishmania* spp., have focused on attraction to humans and domestic animals (1), while data on attraction to wild mammals and feeding success are scarce. The fragmentary information available in the literature has been obtained using Disney traps (2) and blood-meal analysis (3,4). These methods are used to identify mammals that may serve as sources of blood meals for phlebotomines, but in practice both approaches have disadvantages. Disney traps generally require a considerable investment in time and human resources and do not specifically address feeding success; they merely identify those phlebotomines that are most likely to be attracted to the animal that is used as bait. Blood-meal analysis provides data on the source of blood that sand flies utilize, but it does not identify the proportion of sand flies that successfully feed. Furthermore, this method is limited by the number of commercially-available antisera, which frequently do not cover the entire range of mammals to be evaluated, and the cross-reactivity of some of the antisera. The need for freshly-engorged sandflies also presents a serious drawback.

Recognizing that there is no single system for conclusively demonstrating host attraction and feeding success, we used a simple laboratory test to evaluate feeding success of the sandfly *Lutzomyia evansi* Nuñez Tovar, a vector of *Leishmania chagasi* (5,6), on putative small mammal hosts within an endemic focus of visceral leishmaniasis in northern Colombia. This work comple-

ments previous studies on the transmission of *L. chagasi* (5-7) and the ecology of small mammal hosts (8,9) within this focus and provides further evidence for the potential role of two abundant mammals as reservoirs.

Materials and methods

L. evansi females were collected with Shannon traps Shannon (10), and buccal aspirators between 19:00 and 21:00 hours in forested areas of Córdoba and Sucre Departments (Colombia) within the endemic focus of visceral leishmaniasis (6). In this area *L. evansi* represents >95% of the sand fly species composition (6). Fifty unengorged females were introduced into 200 ml plastic containers covered with fine mesh. Mammals were live-trapped in the same area where sand flies were collected, using National traps baited with plantain and peanut butter. Captured mammals were transported to field headquarters and maintained with food and water *ad libitum* until a feeding-success trial was completed (always <24 h). Each trial was conducted between 19:00 and 20:00 hours, within two hours of sand fly collection. The mammals were anesthetized with 70 mg/kg of ketamine hydrochloride (Ketalar®, Parke Davis) and 3 mg/kg of xylazine (Rompun®, Bayer) and placed in a dark room. The fine mesh of the sand fly container was placed in contact with a depilated area of a mammal's hind foot to allow easy access by sand flies to the skin. The area of mesh in contact with the mammal was constant (1 x 3 cm) among all trials to ensure that sand flies in each experiment had equal access, regardless of the size of the mammal. After 30 min, containers were removed and the number of engorged flies recorded; a confirmatory count also was conducted the following morning. To avoid statistical dependence associated with repeated trials on a single individual, each mammal was used only once, ear tagged for proper identification, and then released at its capture site following completion of a trial. Mean numbers of engorged flies were compared among host species with analysis of variance and Tukey's multiple comparisons test.

Results

According to the trapping success in the endemic area, we were able to test 46 individual mammals

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of six species, including the opossums *Didelphis marsupialis* Linnaeus (n=12) and *Marmosa robinsoni* Bangs (n=2) and the rodents *Sciurus granatensis* Humboldt (n=3), *Heteromys anomalus* (Thompson) (n=4), *Zygodontomys brevicauda* (Allen & Chapman) (n=5), and *Proechimys canicollis* (Allen) (n=20). We excluded *M. robinsoni* from analysis because only two individuals were tested (resulting in 28 and 35 engorged sandflies). Mean numbers of engorged flies differed among the five other host species ($F_{4,39}=43.69$, $p=0.0001$). The species on which *L. evansi* fed most successfully were *D. marsupialis* (mean=43.1 engorged flies) and *P. canicollis* (mean=41.7), and the species on which *L. evansi* fed least successfully was *S. granatensis* (mean=2.0) (figure 1). Although sample sizes were small for most species, the small standard errors (1.2 to 3.3) indicated little within-species variation and suggested that the results were robust with respect to sand fly feeding success.

Discussion

Most information on host attraction and feeding success is based on laboratory and field studies of haematophagous arthropods other than sand flies, such as mosquitoes, black flies, and gnats (11-13). These studies have demonstrated that host

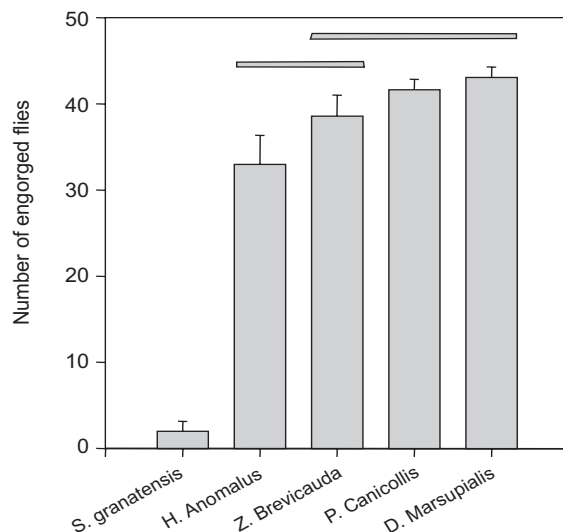


Figure 1. Mean numbers (and standard errors) of engorged sand flies fed on five species of small mammals. Lines at the top of the graph connect species whose means do not differ at $p<0.05$ by Tukey's multiple comparisons test.

attraction and feeding success are the result of a series of stimuli in which CO_2 and odors are crucial cues (11-15).

Host attraction and blood-meal analyses of sand flies of epidemiological importance, such as *Lutzomyia longipalpis*, have shown that feeding patterns can be highly variable depending upon: 1) the site where collections or experiments are made (peri- vs. extradomicile); 2) the availability of individuals on which to feed, and 3) the relative size of the host on which the sand flies potentially could take blood (1,4).

Previous host-attraction and feeding experiments conducted with *L. evansi* in the endemic focus of northern Colombia showed that humans attracted more sand flies than did dogs and opossums (16). However, that study did not control for the different body sizes of the hosts. Our approach, by exposing similar skin areas of the different animals, controlled for size of the hosts but did not address those stimuli that sand flies might use in seeking a host. Rather, our study provided data on the final step of the feeding process (i.e., engorgement), which ultimately determined feeding success. Probing does not necessarily result in successful feeding. In our laboratory, while attempting to use a topical anesthetic to avoid discomfort during xenodiagnosis on humans, *L. longipalpis* probed three different persons repeatedly without feeding. Thus, typical erythema produced by the saliva of *L. longipalpis* were apparent, but sand flies did not engorge.

Our data indicated that, although *L. evansi* females fed on all small mammal hosts in the trials, they showed host-specific differences in feeding success. In particular, *L. evansi* clearly fed least successfully on *S. granatensis*. Although this squirrel is common in the study area, it is unlikely to be important in the transmission of *L. chagasi* because of its diurnal habits and the poor feeding success of *L. evansi*.

L. evansi, as with *L. longipalpis* (1,4), may be an opportunistic feeder (16); therefore, the frequency with which this sand fly species feeds on specific mammals may be related to both their relative abundances and attractiveness to sand flies. It is important to note that *L. evansi* fed most success-

fully on the two most abundant mammals in the study area (*D. marsupialis* and *P. canicollis*), suggesting that a high proportion of the blood meals necessary for maintaining or expanding sandfly populations could be provided by these two primarily-nocturnal species. Because they constitute the greatest biomass of small mammals, and probably of all wild mammals, in the VL endemic area (8) and are known to be naturally infected with *L. chagasi* (7), we hypothesize that *D. marsupialis* and *P. canicollis* are the wild mammals with the greatest impact on transmission of *L. chagasi* in northern Colombia. *D. marsupialis* may be particularly important because of its large home ranges, excellent adaptability to degraded habitats, and frequent proximity to human habitations (9). *H. anomalus* and *Z. brevicauda* are periodically abundant in the study area (8) but are unlikely to assume the importance of *D. marsupialis* and *P. canicollis* as reservoirs of *L. chagasi* because of their lesser contribution to total mammal biomass and the lesser feeding success of *L. evansi*. Furthermore, neither species has yet been found to be infected by *L. chagasi* in this endemic focus.

The present work indicates that, although sophisticated tests are necessary to understand the mechanisms involved in host attraction, simple experiments can help to elucidate sand fly feeding success on putative hosts. When coupled with information on the abundances and ecology of hosts, a better understanding of disease transmission may emerge.

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Foot note: All field and experimental work carried out in this study conformed with the International Guiding Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences and with the Standards for Human Care and Use of

Laboratory Animals. CIDEIM also complies with all applicable provisions of Colombian Law 84 of 1989 in the "Estatuto Nacional de Protección de los Animales". Collecting permits are on file at CIDEIM.